Remarks

In view of the foregoing amendments and the following remarks, reconsideration of the outstanding office action is respectfully requested.

Claims 7, 9, and 49 have been amended, and claim 6 has been cancelled. Descriptive support for the amendments to claim 49 appear in the specification as follows: "derived from poly(2-propenal, 2-propenoic acid) by a process comprising heating poly(2-propenal, 2-propenoic acid) comprising from 0.1 to 5 moles of carboxyl groups per kilogram of polymer in a polyalkylene glycol in the presence of water" is supported by the description appearing on page 7, line 4 to page 8, line 21, Examples 2-4, 8, 9, and 10b, and claim 6 as originally filed; the time period "from 1 to 1400 hours" finds descriptive support at page 14, lines 30-33 and in Example 8; that the derivative polymeric antimicrobial has antimicrobial activity that "is increased compared with the poly(2-propenal, 2-propenoic acid)" finds descriptive support at the paragraph bridging pages 8-9 and Example 11; and the description of forming the starting material poly(2-propenal, 2-propenoic acid) "by homopolymerization of acrolein by ionic initiation and oxidation of the homopolymers of acrolein to introduce said carboxyl groups" is supported at page 5, lines 4-11, page 7, lines 4-6, and Examples 1 and 10a. Therefore, the amendments to claims 7, 9, and 49 do not introduce new matter.

Claims 7, 9-13, 15-17, 25-39, 42, 46, 47, and 49 remain pending.

The rejection of claims 6, 7, 9-13, 15-17, 25-39, 42, 46, 47, and 49 under 35 U.S.C. § 102(b) as anticipated by, or under 35 U.S.C. § 103(a) for obviousness over, PCT Application WO 96/38186 to Melrose ("Melrose") is respectfully traversed.

As noted in applicants' prior submission, Melrose teaches the formation of poly(2-propenal, 2-propenoic acid) from acrolein homopolymers, as well as acrolein copolymers, and their use to treat or prevent gastrointestinal disease in various animals. Melrose merely describes how to form the starting material that can be used to form the polymeric antimicrobial which is administered in accordance with claim 49.

On pages 3 and 6 of the outstanding office action, the U.S. Patent and Trademark Office ("PTO") asserts that Melrose "illustrates a reaction of the subject polymers of poly(2-propenal, 2-propenoic acid) with polyol at room temperature up to 100°C to increase hydrophilicity and utility in the application of treating diseases of the gastrointestinal tract of humans, animals, and birds." In support of this assertion, the PTO cites to page 3, lines 25-35,

page 7, lines 5-15, and Examples 1, 8-11, 13, and 15 of Melrose. For the reasons discussed below, applicants submit that none of these cited portions of the Melrose reference support the PTO's assertion.

In particular, Melrose at page 3, lines 25 to 35, fails to refer to reacting poly(2-propenal, 2-propenoic acid) with a polyol—let alone a polyalkylene glycol as claimed—under the recited conditions. The cited paragraph recites:

> "Particularly, careful heating of the Subject Polymers formed by ionic initiation/catalysis with ample air from room temperature up to 100°C, and preferably up to between 80-85°C, produces the Subject Polymers having 0.1-5 moles of carboxyl groups/kg, aqueous soluble at the pH of the duodenum especially, and preferred for the applications in the gastrointestinal tract described and envisaged herein."

The ionic initiation/catalysis, referred to in lines 25 to 30, relates to the polymerization of acrolein and then its oxidation in air. Thus, this merely describes the formation of the poly(2-propenal, 2-propenoic acid) having 0.1-5 moles of carboxyl groups/kg. As reflected in claim 49, this is the starting material that is used in the derivation process of forming the polymeric antimicrobial as recited.

There is nothing in the disclosure at page 3, lines 25 to 35, of Melrose which involves or requires the use of a polyol, let alone a polyalkylene glycol. A polyol is a compound containing more than one alcohol group; and a polyalkylene glycol is a polymer of an alkylene glycol and has terminal alcohol groups. Methanol is not a polyol and CARBOPOL, which is a polymer of acrylic acid, is not a polyol. Thus, this portion of Melrose fails to disclose heating the poly(2-propenal, 2-propenoic acid) having 0.1-5 moles of carboxyl groups/kg in a polyalkylene glycol in the presence of water at a temperature in the range from 40°C to 150°C for a period from 1 to 1400 hours.

Melrose at page 7, lines 5-15, also fails to mention reacting poly(2-propenal, 2-propenoic acid) with a polyalkylene glycol. Instead, this cited portion of Melrose refers to forming an emulsion containing the poly(2-propenal, 2-propenoic acid) through the use of emulsifying agents, such as natural gums like gum acacia or gum tragacanth, as well as a method of producing pellets of the poly(2-propenal, 2-propenoic acid) with a suitable carrier in a polymeric matrix, i.e., as a controlled release system. Thus, this portion of Melrose also fails to

disclose heating the poly(2-propenal, 2-propenoic acid) having 0.1-5 moles of carboxyl groups/kg in a polyalkylene glycol in the presence of water at a temperature in the range from 40°C to 150°C for a period from 1 to 1400 hours.

Melrose at Example 1 describes the formation of poly(2-propenal, 2-propenoic acid) having 0.1-5 moles of carboxyl groups/kg in two steps. Step (a) recites formation of polyacrolein, and step (b) recites the formation of poly(2-propenal, 2-propenoic acid) having 0.1-5 moles of carboxyl groups/kg by "heat-dr[ying] in contact with ample air, initially at ambient temperatures and then at temperatures up to about 100°C." There is no discussion in Example 1 of Melrose that concerns heating the polymer in a polyalkylene glycol in the presence of water at a temperature in the range from 40°C to 150°C for a period from 1 to 1400 hours.

With regard to Examples 8-11, the PTO asserts on page 4 of the office action that these examples teach "the fixing of poly(2-propenal, 2-propenoic acid), from a homopolymer of acrolein by ionic derivation and oxidation and more specifically…heating at 60-70°C for 48 hours with methanol." This is incorrect for several reasons. Firstly, the initiation catalyst used in Examples 8 to 11 is benzoyl peroxide, which is not an ionic initiator but rather a free radical initiator. Thus, Examples 8-11 do not demonstrate ionic initiation. Secondly, while the polymer in Examples 8 to 11 is said to swell in methanol at 60 to 70°C, methanol is not a polyol, let alone a polyalkylene glycol as presently claimed. Thus, there is likewise no discussion in Example 8-11 of Melrose that concerns heating the poly(2-propenal, 2-propenoic acid) in a polyalkylene glycol in the presence of water at a temperature in the range from 40°C to 150°C for a period from 1 to 1400 hours.

Melrose at Example 13 describes the reaction of benzoyl peroxide (a free radical initiator, as noted above) with a solution of polyethylene glycol acrylate and acrolein in methanol. This reaction describes *copolymerization* of acrolein and polyethylene glycol acrylate, not "polyethylene glycol" as asserted by the PTO. The example does not teach or suggest the *homopolymerization* of acrolein, let alone formation of poly(2-propenal, 2-propenoic acid) having the requisite proportion of carboxyl groups. While polyethylene glycol is a polyol, the compound "polyethylene glycol acrylate", which is referred to in Example 13, is an ester—specifically the acrylate ester of polyethylene glycol—and, accordingly, has only one free alcohol group. Further, as mentioned above, the compound methanol is not a polyol or a polyalkylene glycol. Thus, Melrose at Example 13 fails to teach the production of poly(2-propenal, 2-propenoic acid) that is "formed by homopolymerization of acrolein by ionic

initiation and oxidation of the homopolymer of acrolein to introduce said acid group" as well as "heating the poly(2-propenal, 2-propenoic acid) having 0.1-5 moles of carboxyl groups/kg in a polyalkylene glycol in the presence of water at a temperature in the range from 40°C to 150°C for a period from 1 to 1400 hours."

Citing to Examples 15 and 16 on pages 4 and 10 of the office action, the PTO asserts that these examples "illustrate the subject polymers and CARBOPOL polyol heated to 45C and suggest heating to 90-100C." Applicants disagree, because the PTO has not provided any basis for assuming that CARBOPOL is a polyol. Indeed, this is nowhere to be found in the Melrose application, and that is because CARBOPOL is not a polyol but rather a trade name for an acrylic acid polymer. Example 16 simply demonstrates absorption of the poly(2-propenal, 2-propenoic acid) by the CARBOPOL or ALCOSORB to form a drug delivery system. (Example 15 appears to have been cited mistakenly, as this example relates to the use of poly(2-propenal, 2-propenoic acid) to treat piglets.) Thus, Melrose at Examples 15-16 fails to teach heating the poly(2-propenal, 2-propenoic acid) having 0.1-5 moles of carboxyl groups/kg in a polyalkylene glycol in the presence of water at a temperature in the range from 40°C to 150°C for a period from 1 to 1400 hours.

From the foregoing, it should be appreciated that while Melrose does teach generally the formation of poly(2-propenal, 2-propenoic acid) having 0.1-5 moles of carboxyl groups/kg, and the use of these polymeric materials as an antimicrobial, Melrose fails to teach the "superactivation" of this polymeric material in the manner as presently claimed, where such "superactivation" is achieved by "heating the poly(2-propenal, 2-propenoic acid) having 0.1-5 moles of carboxyl groups/kg in a polyalkylene glycol in the presence of water at a temperature in the range from 40°C to 150°C for a period from 1 to 1400 hours" as recited in claim 49. Because Melrose fails to teach or suggest the making of this "superactivated" form of the polymer, Melrose cannot teach administering the "superactivated" form of the polymer to an animal subject for the treatment of gastrointestinal disease. For this reason, the rejection of claim 49 (as well as claims 7, 9-13, 15-17, 25-39, 42, 46, and 47 dependent thereon) as anticipated by Melrose is improper and should be withdrawn.

The PTO also asserts at pages 9-10 of the outstanding office action that the claimed invention would have been obvious, because Melrose teaches "a reaction of the subject polymers poly(2-propenal, 2-propenoic acid) heated with polyethylene glycol in Example 13 and further teach heating the subject polymers poly(2-propenal, 2-propenoic acid) heated with

CARBOPOL polyol at 45C and suggest heating at 90-100C to increase hydrophilicity and utility in the application of treating diseases of the gastrointestinal tract of humans, animal, and birds." Applicants respectfully disagree.

For substantially the same reasons noted above, applicants submit that the PTO has failed to demonstrate that the recited *derivatives* as recited in claim 49 would have been obvious, and therefore their use could not have been obvious either. One reason why the PTO has failed to establish *prima facie* obviousness is because the asserted foundation upon which the PTO's position rests is factually inaccurate. As noted above, Examples 1 teaches oxidation of poly(2-propenal, 2-propenoic acid) *in air*, and Example 13 teaches *copolymerization* of polyethylene glycol acrylate and acrolein. Example 15 relates only to the use of poly(2-propenal, 2-propenoic acid) to treat piglets, and Example 16 teaches absorption of the poly(2-propenal, 2-propenoic acid) in a CARBOPOL or ALCOSORB polymeric delivery vehicle. None of these examples teaches or suggest "heating the polymer in a polyalkylene glycol in the presence of water at a temperature in the range from 40°C to 150°C for a period from 1 to 1400 hours" as recited in claim 49.

As described in the present application, the formation of the derivative of poly(2-propenal, 2-propenoic acid) in the manner as recited in claim 49 results in a "superactivated" derivative that is structurally distinct of the original poly(2-propenal, 2-propenoic acid) and possesses greater activity that the original poly(2-propenal, 2-propenoic acid). The use of the antimicrobial manufactured in this way has been shown to have a surprising level of activity on treatment of gastrointestinal disease.

In particular, Example 3 of the present application demonstrates that the heating of the poly(2-propenal, 2-propenoic acid) with a polyalkylene glycol (specifically polyethylene glycol) results in the formation of a distinct product. As shown in the NMR analysis on page 25, derivation in the manner as presently claimed results in loss of the δ 5.5 (hydroxyl group) signal due to the formation of protective acetals. Examples 10(a), 10(b) and 11 of the present application specifically demonstrate that the derivative product so made demonstrates a significant increase in antimicrobial activity on gastrointestinal administration, when compared with poly(2-propenal, 2-propenoic acid) of the type disclosed in the prior art. As is evident from comparison of the results in Table 13(a) and 13(b), the improvement in antimicrobial activity, compared with a control, is much more significant when the polymer is heated with polyalkylene glycol. Further, the animals are reported to have a significantly improved weight gain when

compared with compositions of the type reported in the prior art where the polymer has not reacted with polyalkylene glycol.

The difference between the derivative whose use is recited in claim 49 and the prior art poly(2-propenal, 2-propenoic acid) is further demonstrated in Examples 20 and 21 of applicants' co-pending PCT application WO 2003/061672 (copy attached as Exhibit 1), which claims priority from the present application. In comparative Example 20(a), poly(2-propenal, 2-propenoic acid) is formed in accordance with the prior art approach (oxidation in air). In Example 20(b), the polymer is super-activated by reaction with polyalkylene glycol (specifically polyethylene glycol) at 100°C for four hours. Example 21 specifically studies the activity of the two compositions against gastrointestinal disease (cancer cells). The composition of Example 20(b), whose use is recited in claim 49 of the present application, exhibits a significantly improved activity when compared with the prior art poly(2-propenal, 2-propenoic acid).

The present application explains on page 4, lines 12-18, that the derivatives formed by the high temperature reaction between poly(2-propenal, 2-propenoic acid) and the polyalkylene glycol are believed to be acetal or hemiacetal derivatives. The finding that such derivatives have a significantly improved activity in gastrointestinal treatments is surprising, because acetal and hemiacetal groups are renowned as being unstable and would have been expected to be lost in the corrosive environment of the stomach. Accordingly, the surprising improvement in activity could not have been expected from the prior art.

For these reasons, the rejection of claim 49 (as well as claims 7, 9-13, 15-17, 25-39, 42, 46, and 47 dependent thereon) for obviousness over Melrose is also improper and should be withdrawn.

In view of all of the foregoing, applicants submit that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

Date: September 15, 2008

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WO 03/061672 A1

(54) **Title:** METHOD OF TREATMENT OF GASTROINTESTINAL DISEASE AND POLYMERIC COMPOSITION FOR USE THEREIN

(57) **Abstract:** A method of treatment of gastrointestinal disease by administering polymeric antimicrobial comprising a derivative of poly(2-propenal, 2-propenoic acid) formed by reaction between a poly(2-propenal, 2-propenoic acid) and an alcohol or phenol to form protected carbonyl groups. The invention also relates to composition for use in treatment of gastrointestinal disease.

METHOD OF TREATMENT OF GASTROINTESTINAL DISEASE AND POLYMERIC COMPOSITION FOR USE THEREIN

FIELD OF THE INVENTION

The present invention relates to treatment or prophylaxis of gastrointestinal disease and promotion of animal growth and to antimicrobial compositions for use in such treatments.

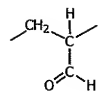
BACKGROUND ART

Antimicrobials are compounds which kill microorganisms, such as bacteria. Antibiotics are a subset of antimicrobials that are (usually) derived from other microorganisms and work by interfering with specific mechanisms within the target microorganism. Antibiotics were first used in the 1940s and 1950s and their use has increased ever since. The development of antibiotic resistance has become a serious and potentially life threatening event worldwide. Some strains of Staphylococcus have shown resistance to almost all antibiotics and have had fatal infection occurring in hospitals. Other drug resistant organisms include pneumococci that cause pneumonia and cryptosporidium and *E.coli* which cause diarrhoea.

The use of antibiotics in animal feed is widely considered to be responsible for the accelerated development of resistance and as a result many countries control their use. This has lead to problems in animal farming resulting in difficulty in controlling disease and obtaining optimum growth rates. This is a particular problem in farming of pigs and poultry. For example, gastrointestinal diseases such as colibacillosis in pigs and coccidiosis in poultry can have a devastating effect.

Melrose et al in (International Patent Publication No. 96/38186) were the first to describe the preparation of acrolein polymers for use in treatment of gastrointestinal disease.

These polymers have a repeating unit of formula I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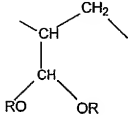
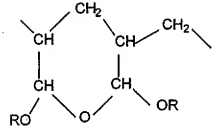
or this unit in its hydrated, hemi-acetal or acetal form, represented by the formulae:


(a)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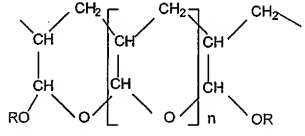
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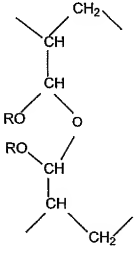
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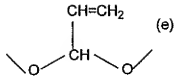
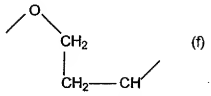
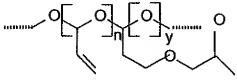




wherein R is hydrogen and n is an integer of one or more, have been demonstrated previously. Prior to this the Biocidal properties of acrolein polymers in antiseptic applications was described by Melrose et al in International Application No. WO88/04671. German Patent Application P4404404 and equivalents such as EP667358 and AU 11686/95 (now lapsed) discloses a process in which acrolein is polymerised in an aqueous sodium hydroxide medium. The disclosure states that the resulting polyacrolein is soluble in polyhydric alcohol at 40 to 50°C to form a solution of the polyacrolein in a polyhydric alcohol. As explained below the assignee of this German application subsequently found such polymers to be problematic and have low solubility in aqueous media.

European publication No. 792895 Werle et al. (corresponding to US 6060571) relates to acrolein releasing polymers prepared by copolymerisation of acrolein monomer and a polyhydric alcohol. Werle et al. observes that the polyacroleins described in German Application No. P4404404 are problematic in that the yield is less than desired and the polymers are virtually insoluble in water. European Application 792895 teaches that these problems are overcome by forming an acrolein releasing polymer by copolymerisation of acrolein monomer and a polyhydric alcohol monomer. The proposed structure of the copolymer is as follows:



While free acrolein acts as an antimicrobial it is irritating to the eyes, lungs, tissues and skin. There is a need in a range of applications particularly in gastrointestinal treatments for antimicrobials which are stable, highly water soluble, and safe to use. There is a further need for an effective antimicrobial for treatment or prevention of gastrointestinal disease, which can reduce the pressure for development of resistance in antibiotics.

The discussion of the background to the invention herein is included to explain the context of the invention. This is not to be taken as an admission that any of the material referred to was published, known or part of the common general knowledge as at the priority date of any of the claims.

Summary of the invention

We have now found the activity and stability of poly(2-propenal, 2-propenoic acid) polymers in treatment or prophylaxis of gastrointestinal disease is substantially increased if they are reacted with an alcohol or polyol to form protected carbonyl groups such as acetal and/or hemiacetal derivatives. Surprisingly we have found that the activity of the derivative in treatment or prophylaxis of gastrointestinal disease is substantially increased notwithstanding that the free acrolein content may be extremely low or negligible in the polymer. The solubility of the polymer in water is also very high.

The invention provides a method of treatment or prophylaxis of gastrointestinal disease in an animal (including humans) comprising administering to the animal an effective amount of a derivative of poly(2-propenal, 2-propanoic acid) formed by reaction between poly(2-propenal, 2-propenoic acid) and an organic compound containing one or more hydroxyl groups such as an alcohol preferably selected from alkanols, phenols, polyols and mixtures thereof, to form protected carbonyl groups.

In a further aspect the invention provides an antimicrobial for treatment of gastrointestinal disease comprising a derivative of poly(2-propenal, 2-propenoic acid) formed by reaction between poly(2-propenal, 2-propenoic acid) and an

organic compound containing one or more hydroxyl groups such as an alcohol preferably selected from alkanols, phenols, polyols and mixtures thereof, to form protected carbonyl groups.

The term polyol as used herein means a molecule containing at least two hydroxyl groups.

The derivatives formed are typically selected from hemiacetal and acetal derivatives. Without wishing to be bound by theory we believe that the reaction of the poly(2-propenal, 2-propenoic acid) with the alcohol forms hemiacetal and/or acetal groups from at least a proportion of the pendent aldehyde groups thereby stabilising the carbonyl groups of the polymers against alkaline degradation by the Cannizzaro reaction. The formation of acetal groups has been found to significantly reduce or eliminate the release of free acrolein while surprisingly increasing the activity of the resulting derivative.

In yet another embodiment the invention provides the use of the above described antimicrobial for preparation of a medicament for treatment or prophylaxis of gastrointestinal disease.

Throughout the description and claims of this specification, the word "comprise" and variations of the word such as "comprising" and "comprises", is not intended to exclude other additives or components or integers.

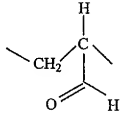
Detailed Description Of The Invention

The antimicrobial of the invention may be prepared by heating poly(2-propenal, 2-propenoic acid) in the presence of the alcohol, preferably a polyol such as polyethylene glycol. Water is invariably present in the alcohols and it will be understood that the presence of at least some water assists in the nucleophilic reaction resulting in hemiacetal or acetal formation.

The solution is generally heated at a temperature in the range of from 40°C to 150°C, more preferably 40 to 115°C and most preferably 70 to 115°C.

The antimicrobial of the invention is prepared from poly(2-propenal, 2-propenoic acid) polymers. Such polymers and their preparation are described in International Patent Publication No. WO 96/38186 (PCT/AU96/00328) the contents of which are herein incorporated by reference. The poly(2-propenal, 2-propenoic acid) polymers are preferably prepared by polymerisation of acrolein preferably in aqueous solution by anionic polymerisation, followed by autoxidation. The polymers contain the repeating unit of formula I and at least one (and typically a mixture) of the hydrated, hemiacetal and acetal forms.

The hydrated, hemiacetal and acetal forms formed by polymerisation of acrolein are known to arise from the various carbon-carbon and carbon-oxygen polymerisation mechanisms of acrolein. For example the hydrated form is typically the hydrated diol form, the hemiacetal or acetal form may be formed from the condensation of the diol form with the aldehyde or diol form, the tetrahydropyran or fused tetrahydropyran form may be formed from condensation of the diol form and the aldol-Michael self condensation form. Typical examples of these forms are shown in formula (a) to (f) below:



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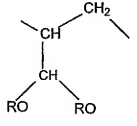
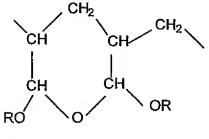


(a)



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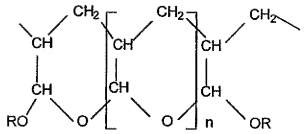

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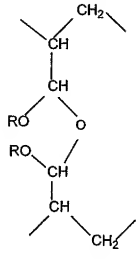

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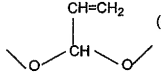

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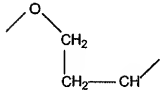

(f)

wherein R is hydrogen and n is an integer of one or more. The proportion of repeating unit of formula I is typically less than 20% and frequently from 5 to 15%. Notwithstanding the relatively low proportion of these units we have found that they have a significant effect on the stability of the polymer.

The poly(2-propenal, 2-propenoic acid) will generally contain no more than 10% on a molar basis of monomer units from monomers other than acrolein and is most preferably an acrolein homopolymer (before autoxidation). Where used other monomers may be selected from the group consisting of acrylic acid and

vinyl pyrrolidone. The 2-propenoic acid groups are typically present in an amount of from 0.1 to 5 moles of carboxyl groups per kilogram. The poly(2-propenal, 2-propenoic acid) polymers typically have a number average molecular weight of over 1000 and most preferably over 2000. Typically the molecular weight is less than 10,000.

The antimicrobial of the invention is a derivative of poly(2-propenal, 2-propenoic acid) prepared by reaction with an alcohol or phenol to form protected carbonyl groups. The protected carbonyl groups are formed from the 2-propenal groups, which react with the alcohol to form hemiacetal and acetal groups. The alcohol is preferably a polyol by which is meant that it preferably contains at least two hydroxyl groups. Alkanols such as C_1 to C_{10} may be used. Where the alcohol is a polyol the reaction may produce acetals or hemiacetals formed by reaction of one or more than one alcohol group. Furthermore when two alcohol groups react it is possible for them to react at the same carbonyl or different carbonyl groups within the polymer.

Referring to the above formula I and hemiacetal and acetal forms the invention produces derivatives in which there are fewer units of formula I and forms a group wherein one or more groups R are derived from an alcohol, or when the alcohol is a polyol, more than two groups R may together form a bridging group such as a cyclic acetal group.

The propensity for polyols to give rise to internal cyclic groups will depend on the spacing and configuration of the polyol. The preferred alcohols are polyalkylene glycols and more preferred alcohols are polyethylene glycols.

The molecular weight of the polyalkylene glycols is preferably from 200 to 2000 and more preferably from 200 to 1000.

Preferably, the alcohol such as polyethylene glycol is present during the preparation of the antimicrobial polymers in an amount of between 50 and 99% by weight. Relatively dilute compositions of the acrolein polymer are particularly

preferred where the alcohol is a polyol as the incidence of intermolecular cross-linking is reduced by dilution.

More preferably, polyethylene glycol is present during preparation of the polymers in the amount of between 64 and 95% by weight.

Base or alkali is preferably added to the polymers followed by a drift to acidic pH before and/or during heating, as neutralization of the acid groups of the polymer occurs, thereby enhancing the antimicrobial activity of the polymers. Preferably, the addition of the base or alkali initially brings the pH of the poly(2-propenal, 2-propenoic acid) polymers to between 7 and 9. Still more preferably, the initial pH on addition of the base is about 8. The base is preferably an alkali metal hydroxide, carbonate, bicarbonate or mixture thereof.

In a still further form of the invention, the release of free acrolein monomer is inhibited, from continuous release, whereby the polymers are less likely to present a source of tissue or dermal irritation.

We have found that the antimicrobial of the invention has significantly improved activity in controlling gastrointestinal disease when compared with the poly(2-propenal,2-propenoic) from which it is prepared. The superactivated derivative of the present invention may be used to treat a wide range of animals (including humans) and a wide range of microbial infections.

The antimicrobial of the invention may be used in treatment of gastrointestinal disease in humans, however it is particularly preferred that it be used in treatment of other animals particularly animals selected from the group consisting of dogs, pigs, sheep, horses, goats, cattle, cats, poultry, ducks, turkeys and quail.

The antimicrobial of the invention may be formulated for oral or rectal administration. Rectal administration may be particularly useful in ruminant animals. Oral formulations for ruminant animals may also be prepared using

enteric coatings to provide optimal activity in the later part of the gastrointestinal tract.

The antimicrobial of the invention is particularly useful in treatment and prophylaxis of gastrointestinal ulcers, diarrhoea and gastrointestinal cancers. The antimicrobial of the invention may also be used to improve the rate of weight gain in farm animals by improving the feed to weight conversion in animals.

We have found that the antimicrobial of the invention may be used as a growth promotant and that the polymer may be used in place of the presently used antibiotics. Drug resistance in pathogenic bacteria is a problem of major clinical importance in human medicine. This problem is exacerbated by the use of important antibiotics in animal feed to provide weight gain in farm animals particularly poultry and pigs. Indeed, in some European countries the use of conventional antibiotics in animal feeds has been banned. The antimicrobial of the invention may be used in treatment of animals to significantly extend the useful life of conventional antibiotics in human treatment.

We have found the antimicrobial of the invention to be effective against a wide range of microbes including protozoa, Gram positive bacteria and Gram negative bacteria. The polymers of the invention contain multiple structures of diverse configurations and can find a fit with the proteins found in the cell wall of target organisms, this speeds up the inactivation of the protein and the destruction of the cell. Of the Gram negative bacteria the antimicrobial of the invention has been found to be particularly useful in providing broad spectrum activity against coliforms or Enterobacteria. It is particularly useful in treatment of gastrointestinal diseases resulting from infection by *E. coli* such as enterotoxigenic *E. coli* and β-haemolytic *E. coli*. Colibacillosis is a devastating disease in the pig-rearing industry. The disease is generally associated with proliferation of β-haemolytic *E. coli*. in the small intestine after weaning and gives rise to high mortality rates and morbidity rates in young weaner piglets. Infected weaner piglets fail to make normal weight gains.

Coccidiosis is a protozoal disease of animals particularly poultry and if left uncontrolled has a devastating effect. We have found that the antimicrobial of the invention may be used in the treatment or prevention of coccidiosis in birds particularly in poultry. In chickens typical clinical signs of coccidiosis include lack of thriving, rapid loss of weight, diarrhoea and dysentery. The most serious effects take place in the intestine where the protozoa tend to invade the mucosa and cause epithelial damage, lesions and haemorrhage. Vaccines have been used in an attempt to prevent coccidiosis but have side effects including the tendency to reduce weight and feed efficiency.

The antimicrobial of the invention may be used in combination with other drugs known to have activity against coccidiosis. Such drugs include nitro-carbanilide, quinoline, pyridon, guanidine, quinoxaline, toltrazural, toluamide, potentiated sulfa drugs and ionophore with carbanilide.

Clostridia are Gram positive bacteria responsible for serious disease in a range of animals. For example, necrotic enteritis is a disease known to affect commercial poultry. Clostridia bacterial produce exotoxins which are some of the most toxic of all known toxins. Necrotic enteritis particularly effects broilers of between 14 and 42 days of age. The condition causes pronounced apathy, diarrhoea and can cause death within hours.

Upper gastrointestinal disease including chronic gastritis, gastric ulcer and duodenal ulcer are significant human health problems. *Helicobacter* is understood to be responsible for the development of ulcers and the development of gastrointestinal cancers particularly adenocarcinoma of the stomach. We have found the antimicrobial of the invention to be particularly useful against *Helicobacter* including *H. pylori* in gastrointestinal disease in animals, particularly humans.

The infection of the stomach with *Helicobacter pylori* is one of the most frequent infectious diseases in the world. About 50% of the population are infected *with H. pylori.* In developing countries it has been estimated that more than 80% of the population is already infected with *H.pylori* during childhood.

Helicobacter pylori is a Gram-negative, microaerophilic, spiral-shaped, bacilli that is motile by way of flagella at one end of the cell. The standard treatments of *H. pylori* infections are the so-called triple antibiotic therapies all of which include either metronidazole or clarithromycin. Unfortunately strains of *H. pylori* have emerged, which are resistant to both these antibiotics.

H. pylori live in the stomach at the interface between the surface of gastric epithelial cells and the overlying mucus gel layer. *H. pylori* can additionally be found on top of the gastric epithelium in the duodenum and oesophagus. Other animal species have their own unique *Helicobacter* species present in their gastrointestinal tracts, which have similar properties to *H. pylori*. In addition to its association with gastrointestinal cancers, *H. pylori* has been directly linked in humans to gastritis and peptic ulcer formation.

Helicobacter species in general, and *H. pylori* in particular, survive the extreme conditions of the stomach by secreting urease, which hydrolyses urea to give ammonia and bicarbonate ion, thus raising the pH of the immediate surroundings of the bacilli. This local alteration of the conditions protects the bacteria from the bactericidal effect of the gastric acid. The preferred position underneath the stomach's protective mucus layer is also a survival advantage and its motility allows it to burrow through the layer to attain this position.

The epithelial cells lining the stomach are naturally difficult to penetrate; this is part of their function to protect the rest of the body from gastric acid and digestive juices. This difficulty in penetration also makes it difficult for the body's natural defences to pass through the stomach wall and reach the site of *H. pylori* infection. This has two consequences; the body sends more nutrients to the site to aid the white cells, T-cells, and other defence mechanisms, concomitantly supplying the bacilli; and the defence cells eventually die, releasing their cargo of superoxide ion and other lethal chemicals, damaging the surrounding epithelial cells.

It is apparently this activity that leads to gastritis, which can easily progress to peptic ulcers. Should the insult continue, the possibility of the appearance of gastric adenocarcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma is greatly increased. Gastric adenocarcinoma begins in the mucosa and the first stage of development, intestinal metaplasia, is a response of the stomach to rid itself of the *H. pylori* infection. Also, studies performed in UCL Medical School have shown that the MALT lymphoma requires help from *H. pylori* specific T-cells to grow. Treatment of *H. pylori* infection has been shown to be extremely effective in curing MALT lymphoma. The World Health Organisation has labelled the pathogen a Group I carcinogen.

Accordingly the present invention also provides for a method for the treatment or prophylaxis of diseases of the gastrointestinal tract caused by *Helicobacter* infection comprising the gastrointestinal administration of a therapeutic amount of an agent wherein the agent comprises a derivative of poly(2-propenal, 2-propenoic acid) formed by the reaction between a poly(2-propenal, 2-propenoic acid) and an organic compound containing hydroxyl groups selected from alkanols, phenols, polyols and mixtures thereof, to form protected carbonyl groups. The term polyol where used herein means a compound containing at least two hydroxyl groups. The derivatives formed are typically selected from hemiacetal and acetal derivatives

Hence, the use of the method of the present invention provides an alternative to the use of surgery, radiation therapy or traditional chemotherapy in the treatment of gastrointestinal cancers.

The invention further provides a method of treatment for gastrointestinal infection by a species of *Helicobacter* bacteria such as gastritis, gastric ulcer, duodenal ulcer, gastric malignant lymphoma or gastric cancer, comprising the gastrointestinal administration of a therapeutic amount of an agent wherein the agent comprises a derivative of poly(2-propenal, 2-propenoic acid) formed by

the reaction between a poly(2-propenal, 2-propenoic acid) and an organic compound containing one or more hydroxyl groups to form protected carbonyl groups.

The present invention provides an alternative to standard treatments of *Helicobacter* infections, which, in general, comprise the so-called triple antibiotic therapies all of which include either metronidazole or clarithromycin. Strains of *H. pylori* have emerged which are resistant to both these antibiotics and we have shown that the method of the present invention can effectively treat such antibiotic resistant bacteria.

The agent which is a product of the reaction between poly(2-propenal, 2-propenoic acid) and an organic compound containing one or more hydroxyl groups has been shown to be more effective in the treatment of *Helicobacter* infections than the corresponding non-superactivated poly(2-propenal, 2-propenoic acid) groups.

The invention further provides the use of a derivative of poly(2-propenal, 2-propenoic acid) in manufacture of a medicament for treatment or prophylaxis of a disease caused by *Helicobacter* infection.

The method of the present invention may be used in treatment or prophylaxis of gastrointestinal cancers. These may include, for example, cancers of the oesophagus, stomach, intestine and colon. An example of such a type of cancer is the human colon cancer cell line HT-29.

When the antimicrobial of the invention is incorporated into an animal feed or water this may be done in the usual manner. In a preferred embodiment the antimicrobial of the invention is incorporated in a premix. The premix will preferably include the antimicrobial, a physiologically acceptable carrier and optionally a feedstuff. The premix is generally in a relatively concentrated form and is adapted to be diluted with other material such as one or more of the

other carriers, vitamins and mineral supplements and feedstuff to form the final animal feed. The premix preferably includes the antimicrobial in a concentration in the range of from 0.1 to 70% by weight, preferably 0.5 to 50% by weight. The optimum concentration will depend on whether the treatment is preventative, for control or remedial and whether the antimicrobial of the invention is the only active or whether it is used in concomitant therapy with other materials or antimicrobials.

In a preferred embodiment the concentrated composition of the antimicrobial is in a controlled-release form. The controlled release form will include the antimicrobial and a polymeric material for providing controlled release of the antimicrobial from the controlled-release system and is particularly useful in compositions for addition to solid feed material. As a result of the controlled release formulation the release of the antimicrobial may be delayed so as to occur mainly in the duodenum. A controlled release polymer may also minimise rejection of the composition due to taste or be used for rectal suppositories.

An antimicrobial composition in accordance with the invention may be in the form of pellets, pills or like solid composition. The pellets containing the antimicrobial of the invention may be prepared by the steps of:

(i) dissolving said antimicrobial in an aqueous alkaline or basic solution;

(ii) neutralising said solution with acid;

(iii) adding to said neutralised solution insoluble, cross-linked, absorbent polymers of acrylic acid and/or copolymers of acrylamide and acrylic acid, to form wet swollen pellets; and

(iv) optionally, wholly or partially drying said wet swollen pellets.

The so-formed wet, swollen pellets may be used either wet, partially dried or wholly dried, as an additive to, for example, animal feed. This system is further designed so that the carboxyl-containing groups of the outer polymeric matrix cause the Subject Polymers to remain essentially contained within the matrix when in the acidic environment of the stomach. However, in the alkaline environment of the duodenum, the carboxyl groups of the matrix become

ionised and mutually-repelling, and the pellet rapidly swells to allow the Subject Polymers, aided by repulsion among their own ionic groups, to be excluded by a diffusion process, approximately matching the speed of passage of feed through the duodenum.

In this invention, the term, "controlled release system" is used in the same context as that in, and includes the same range of examples as quoted in "Controlled Drug Delivery" (Robinson & Lee, 1987). Many other pH-sensitive controlled-release systems which are known in the art (Robinson and Lee, 1987) may be substituted for the polymer of acrylic acid or copolymer of acrylamide and acrylic acid. For example, soluble and anionic, or insoluble cross-linked and anionic, cellulosic systems; or soluble and anionic, or insoluble cross-linked and anionic polymers derived from any generic acrylic acid polymer and/or its derivatives. Such cross-linked and insoluble polymers are preferred since they swell and also are less likely to be metabolised.

It is preferred that the controlled release system comprises a pH-sensitive, cross-linked, water-absorbent pellet, which when wet is a gel.

The invention also provides an animal feed composition comprising the antimicrobial of the invention and a feedstuff. The antimicrobial is preferably present in an amount of from 0.0001 to 25% of the total feed composition and preferably from 0.0001 to 5% of the total feed composition.

In another preferred embodiment, the antimicrobial of the invention may be formulated for addition to the drinking water of animals.

The antimicrobial of the invention is preferably administered in amounts of from 0.05 to 5000 mg/kg of bodyweight/day more preferably from 0.05 to 50 mg/kg/day.

Examples of suitable inert carriers for use in compositions for administration of the antimicrobial of the invention include water, olive oil, peanut oil, sesame oil, sunflower oil, safflower oil, arachis oil, coconut oil, liquid paraffin, ethylene

glycol, propylene glycol, polyethylene glycol, ethanol, propanol, isopropanol, glycerol, fatty alcohols, triglycerides, polyvinyl alcohol, partially hydrolysed polyvinyl acetate and mixtures thereof.

Solid forms for oral or rectal administration may contain pharmaceutically or veterinarally acceptable binders, sweeteners, disintegrating agents, diluents, flavourings, coating agents, preservatives, lubricants and/or time delay agents. Suitable binders include gum acacia, gelatine, corn starch, gum tragacanth, sodium alginate, carboxymethylcellulose or polyethylene glycol. Suitable sweeteners include sucrose, lactose, glucose or flavonoide glycosides such as neohesperidine dihydrochalcone. Suitable disintegrating agents include corn starch, methylcellulose, polyvinylpyrrolidone, xanthan gum, bentonite, alginic acid or agar. Suitable diluents include lactose, sorbitol, mannitol, dextrose, kaolin, cellulose, calcium carbonate, calcium silicate or dicalcium phosphate. Suitable flavouring agents include peppermint oil, oil of wintergreen, cherry, orange or raspberry flavourings. Suitable coating agents include polymers or copolymers of acrylic acid and/or methacrylic acid and/or their esters, and/or their amides, waxes, fatty alcohols, zein, shellac or gluten. Suitable preservatives include sodium benzoate, vitamin E, α-tocopherol, ascorbic acid, methyl parabens, propyl parabens or sodium bisulphite. Suitable lubricants include magnesium stearate, stearic acid, sodium oleate, sodium chloride or talc. Suitable time delay agents include glyceryl monostearate or glyceryl distearate.

Suspensions for oral or rectal administration may further comprise dispersing agents and/or suspending agents. Suitable suspending agents include sodium carboxylmethylcellulose, methylcellulose, hydroxypropylmethylcellulose, polyvinyl-pyrrolidone, sodium alginate or cetyl alcohol. Suitable dispersing agents include lecithin, polyoxyethylene esters or fatty acids such as stearic acid, polyoxyethylene sorbitol mono- or di-oleate, -stearate or –laurate, polyoxyethylene sorbitan mono- or di-oleate, -stearate or –laurate and the like.

The composition of the antimicrobial may further comprise one or more emulsifying agents. Suitable emulsifying agents include dispersing agents as exemplified above or natural gums such as gum acacia or gum tragacanth.

Compositions for administration in the method of the invention may be prepared by means known in the art for the preparation of compositions (such as in the art of veterinary and pharmaceutical compositions) including blending, grinding, homogenising, suspending, dissolving, emulsifying, dispersing and where appropriate, mixing of the Subject Polymers together with selected excipients, diluents, carriers and adjuvants.

For oral administration, the pharmaceutical or veterinary composition may be in the form of tablets, lozenges, pills, troches, capsules, elixirs, powders, including lyophilised powders, solutions, granules, suspensions, emulsions, syrups and tinctures. Slow-release, or delayed-release, forms may also be prepared, for example in the form of coated particles, multi-layer tablets or microgranules.

It is generally preferred that poly(2-propenal, 2-propenoic acid) is prepared from poly(2-propenal) by oxidation of the solid in air. The poly(2-propenal) polymer may be initially heated, predominantly in the dry state, to between 80 and 110°C. More preferably, the polymer is initially heated to about 85°C. The poly(2-propenal, 2-propenoic acid) is preferably heated in the alcohol for a period in the range of from 1 hour to 1400 hours and more preferably from 1 hour to 60 hours.

In accordance with the present invention there is further provided a preservative compound or composition comprising the antimicrobial of the invention.

In accordance with the present invention there is yet still further provided a disinfectant or antiseptic compound or composition comprising the antimicrobial of the invention.

In accordance with a further aspect of the invention we provide a composition for treatment of gastrointestinal disease comprising an antimicrobial polymer as hereinbefore described and a further chemotherapeutic agent wherein the further chemotherapeutic agent is adsorbed onto the antimicrobial.

The adsorption will typically reduce membrane penetration of the further chemotherapeutic. The suitable chemotherapeutics for use in this embodiment are those which exhibit a significant reduction in membrane penetration when admixed with the polymeric antimicrobial. Preferably the penetration is inhibited by a factor of at least 50%.

The useful chemotherapeutic agents for use in this aspect of the invention include antibiotics for treatment of gastrointestinal disease and anticancer agents for treatment of gastrointestinal cancers.

The use of chemotherapeutics in combination with the polymeric antimicrobial reduces membrane penetration of the chemotherapeutic thereby reducing systemic side effects and providing more targeted therapy. In many cases odour is also reduced.

Examples of chemotherapeutics for treatment of gastrointestinal disease include antibiotics and anticancer agents.

Examples of antibiotics which may be used in combination with the antimicrobial polymer include tetracyclines, penicillins, aminoglycosides, sulfa drugs, cephalosporins and nitrofurans.

The antibiotics may be conventional antibiotics used to treat infections of the gastrointestinal tract.

Examples of anticancer agents which may be used in combination with the polymeric antimicrobial of the invention are alkylating agents, antimetabolites, anticancer antibiotics, plant alkaloids, hormones and other anticancer agents, particularly anticancer agents containing carbon, hydrogen and oxygen only.

The compositions of the invention may also comprise one or more further antimicrobials such as those selected from the group of a phenol (preferably in an amount of 0.1 to 10% by weight), an isothiazolinone (preferably in an amount of fromk 0.001 to 1%), an alkyl parabens (preferably in an amount of from 0.02 to 2%) and a lower alcohol (preferably in an amount of from 20 to 99%) wherein the amounts are on the basis of weight by weight of the composition.

The derivative of poly(2-propenal, 2-propenoic acid used in the method of the invention has been found to have significantly increased stability compared with of poly(2-propenal, 2-propenoic acid) polymers. Since the prior art recorded some instability of poly(2-propenal, 2-propenoic acid), as evidenced by loss of antimicrobial activity of its compositions, we conducted "accelerated ageing" at elevated temperature, ie. at 40°C. However, to our greatest surprise, the elevated temperature of "ageing" poly(2-propenal, 2-propenoic acid) in aqueous or in aqueous-polyethylene glycol solutions at 40°C, not only slowed the decrease in antimicrobial activity-but in fact, actually increased antimicrobial activity of the poly(2-propenal, 2-propenoic acid), see Example 2(a) and (b). This finding is totally contradictory and unexpected in view of the prior art which predicts that the rise in temperature should lead to "accelerated ageing", ie. accelerated loss of antimicrobial activity.

Herein, the process of providing increased antimicrobial activity by the formation of a new configuration of the subject polymers including poly(2-propenal, 2-propenoic acid), is referred to as "super-activation" and the polymers referred to as "super-activated polymers".

Even more surprising, in view of prior art, the inventors have found superactivation in aqueous polyethylene glycol solution is promoted by basic, followed by acidic conditions. Also, super-activation is promoted by heat and moisture.

Super-activation is facilitated by the presence of polyethylene glycols or polyols or alkanols, we believe, since the presence of the polyethylene glycol or polyol

or alkanol protects and stabilises the carbonyl groups of the polymers, by formation of acetals, from alkaline degradation by the Cannizzaro reaction.

An added advantage of super-activation is that it reduces or eliminates, contaminant acrolein which is a source of tissue and dermal irritation.

It is emphasised that super-activation is quite distinct and additional to any increase of antimicrobial activity which may result, merely from more polymer being available in any aqueous test-medium as the result of increased hydrophilicity of the polymer such as was demonstrated in lapsed Australian Patent Application AU-A-11686/95 (hereinafter "11686/95"). The inventors have repeated exactly the method described in 11686/95 and then, following, found that subsequent super-activation of the partially soluble polymer demonstratively gave rise to additional, substantial antimicrobial activity. It should be noted that even super-activation did not render the polymer from 11686/95 completely soluble-in contrast to super-activation beginning with polymer firstly heated between to 80-85°C.

The optimum time to achieve super-activation of solutions of poly(2-propenal, 2-propenoic acid) depends inversely upon the temperature. It will be apparent that even ageing at room temperature may be used for superactivation, especially when facilitated in the presence of hydroxylic solvent and/or base followed by acidity, but obviously, this may be impractical due to the longer time periods required.

The inventors have found polymers super-activated as described herein, suitable for gastrointestinal therapy, preservatives in water-based products or processes, and active ingredients in disinfectants or antiseptics having the advantage of enhanced antimicrobial activity. Furthermore, the inventors found that the antimicrobial activity of such disinfectants or antiseptics was increased by increase in their pH, for example above pH 6.

A common feature of the invention is the attachment of a group capable of hydrophobic interaction, to the antimicrobial of the invention, by way of

hemiacetal/acetal formation or by way of adsorption, in order to enhance antimicrobial activity.

The invention will now be described with reference to several Examples, which should not be construed as limiting the scope thereof.

BIOCIDAL TEST

Dissolve sample with 1% by weight aqueous sodium bicarbonate to obtain the required concentration (unless specified to the contrary, 0.125% by weight of polymer). Weigh 19.9g of diluted sample into a sterile jar and inoculate with 0.1 mL of 10^7-10^8 cfu of *Ps.aeruginosa* and mix. At specified time-intervals, transfer 1 mL of inoculated sample to 9 mL of Letheen broth and vortex. Plate out serial 1 in 10 dilutions. Pour with trypticase soy agar. Incubate 3 days at 37°C.

Example 1

The example describes a method of preparing poly(2-propenal, 2-propenoic acid) by oxidation of a solid acrolein polymer in air. This poly(2-propenal, 2-propenoic acid) is the preferred method of preparing a starting material for use in the method of the invention. Water (720 mL at ambient temperature, about 20°C) and acrolein (60g ; freshly distilled, plus hydroquinone added to 0.25% w/w) were placed in an open beaker, within a fume cupboard, and very vigorously stirred, mechanically. Then, 0.2 M aqueous sodium hydroxide (21.4 mL) was added to bring the pH to 10.5-11.0.

The solution immediately turned a yellow typical of the hydroquinone anion and within a minute, the colour had disappeared and the clear solution became milky.

About 1 minute later, precipitation of a white flocculent polymer began, and appeared complete within 15-30 minutes. The precipitate was filtered and washed with water (250 mL), dried at room temperature upon filter papers for 2 days (yield 25g), then spread as a thin layer in glass petri dishes and heated at 40° C/8 hours. This heating was continued at the following schedules : 50° C/15 hours ; 65° C/4 hours ; 75° C/18 hours ; 84° C/24 hours.

It is envisaged that this method may be scaled-up to include, eg the stepwise addition of acrolein, in a closed vessel, and followed by more rapid drying (compare example 10).

Typically, a solution of the resulting poly(2-propenal, 2-propenoic acid) was prepared by adding 2g of the subject polymer, with stirring over 15-30 minutes, to a 1% w/w aqueous sodium carbonate solution (100 mL), and then diluted as required. Such solutions were perfectly clear-in contrast to attempted dissolutions, using alternatively, polymer derived from Example 5 of 11686/95.

Example 2

This example describes acetal formation from poly(2-propenal, 2-propenoic acid).

(a) 5g of poly(2-propenal, 2-propenoic acid) was dissolved in 64g polyethylene glycol ("PEG") 200 and combined with 31g of a 0.71 % w/w solution of sodium carbonate. A portion of the solution (apparent pH=5.8) was retained at room temperature while

(b) the remainder of the sample from part (a) was heated at 60° C for periods of 12 or 25 days.

Samples from (a) and (b) were diluted with 1% w/w sodium bicarbonate and submitted for biocidal testing at polymer concentrations of 0.125% w/w. Surprisingly, the samples which had undergone "accelerated ageing" showed improved antimicrobial activity, as can be seen by reference to Table 1:

Table 1

	Cfu/mL * *(Pseudomonas aeruginosa)*				
Sample	0 min	10 min	15 min	30 min	60 min
25 days at room temperature	$7.8x10^6$	$4.1x\ 10^6$	$6.1x10^5$	$9.8x10^4$	<10
12 days at 60°C	$7.7x10^6$	$1.4x10^6$	$9.8x10^3$	<10	<10
25 days at 60°C	$1.0X10^7$	$1.3X10^6$	$6.6X10^4$	<10	<10

* Colony forming units/mL

1g poly(2-propenal, 2-propenoic acid) was dissolved in 200 mL of 0.1% w/w Na_2CO_3 and allowed to stand overnight. Sodium lauryl sulphate was introduced at a level of 0. 05% w/w and the solution was acidified with HCl to pH 5.9. Portions were stored at both room temperature and 60° C. Biocidal Tests were carried out on 0.125% w/w polymer solutions, with 1% w/w $NaHCO_3$ used as the diluent. The "aged" sample showed a surprising improvement in performance, as can be seen by reference to Table 2:

Table 2

	Cfu/mL * *(Pseudomonas aeruginosa)*			
Sample	0 min	10 min	15 min	30 min
20 days at room temperature (RT)	$9.0x10^6$	$5.1x\ 10^5$	$6.8x10^2$	<10
7 days at 60°C + 13 days at RT	$9.0x10^6$	$1.2x10^2$	<10	<10

* Colony forming units/mL

(c) A 5% w/w solution of super-activated polymer was prepared as in example (2a) but replacing PEG200 with PEG1000. A portion of this solution was treated with conc. NaOH to pH 8.1. Samples were heated at 60° C and submitted for biocidal testing. The sample exposed to more basic conditions, unexpectedly gave superior biocidal performance, as can be seen by reference to Table 3:

Table 3

	Cfu/mL * *(Pseudomonas aeruginosa)*				
Sample	0 min	5 min	10 min	15 min	30 min
pH 5.8, 12 days 60°C	$3.8x10^6$	$2.7x10^6$	$1.5x10^6$	$3.3x10^3$	<10
pH 8.1, 7 days 60°C	$90X10^6$	-	10	<10	<10
pH 8.1, 17 days 60°C	$8.3x10^6$	$3.3x10^5$	$1.3x10^2$	<10	<10

* Colony forming units/mL

Example 3

This example examines the product produced by reaction of the poly(2-propenal, 2-propenoic acid) with polyethylene glycol.

The presence of acetals in the polymers of Example 2(b) may be determined by examining the solid residue left after dialysis and concentration of the polymer solution using proton (^{1}H) and carbon (^{13}C) NMR spectroscopy. Dialysis removes all material of molecular weight less than a 1000. Table 1 provides proton (^{1}H) and carbon (^{13}C) NMR data. As can be seen from Table 1, Nuclear Magnetic Resonance spectroscopy of the residue showed peaks at δ 3.58 and 3.56 in the ^{1}H Nuclear Magnetic Resonance spectrum and δ 71.62, 69.48 and 60.25 in the ^{13}C Nuclear Magnetic Resonance spectrum. These peaks are indicative of the attachment of polyethylene glycol units as acetals.

Table 4

Data from the 600 MHz ^{1}H- and 125MHz ^{13}C-Nuclear Magnetic Resonance Spectra in D_2O with 1% w/w Na_2CO_3 of the solid residue of Superactivated polymer after dialysis and concentration.

Region	carbonyl	alkenyl	vinyl methine	vinyl methylene	acetals	oxy-alkyls	PEG	Alkyls
^{1}H δ	9.5-8.5	7.3-6.0	6.0-5.5	5.5-5.0	5.0-4.0	4.0-3.0	3.58, 3.56	3.0-0.5
^{13}C δ	185-175	140-105			105-95	80-55	71.62, 69.48, 60.25	55-15

Example 4

(a) 5% w/w solutions of polymers of a range of degrees of super-activation, apparent pH 5.7, were prepared similarity to Example 2(a), but varying the percentage of PEG 200.

Samples were heated at 60° C and stabilities were monitored over time. Physical stability was considered to have failed with the occurrence of precipitation or gelling. UV measurements were made on a 0.01% w/w polymer

concentration in 1% w/w sodium carbonate solution. A decrease of the ratio of absorption at 268 nm : 230 nm is considered synonymous with a decrease in chemical stability. Results are shown in Table 5:

Table 5

Composition	A	B	C	D
PEG 200 (% by weight)	0	50	64	95
Physical Stability				
Time	A	B	C	D
4 days 60° C	Fail	Pass	Pass	Pass
11 days 60° C	Fail	Fail	Pass	Pass
Chemical Stability	Ratio = 260-270 peak absorbance / 228-235 peak absorbance			
Time	A	B	C	D
0 days 60° C	1.38	1.41	1.43	1.46
4 days 60° C	0.98	1.04	1.21	1.27
11 days 60° C	-	0.97	1.03	1.09
18 days 60° C	-	0.89	0.92	1.04
25 days 60° C	-	-	0.84	1.04

Both physical and UV spectral results demonstrate the positive effect of PEG on stability; higher PEG content results in greater physical and chemical stabilities.

(b) The following solutions A and B were prepared by dissolving 4g of poly(2-propenal, 2-propenoic acid) in 196g 1% w/w sodium bicarbonate and adjusting the pH to 7 (A) and 5.5 (B) with dilute HCl. Solution C was prepared by dissolving 50 g of poly(2-propenal, 2-propenoic acid) in PEG 200 (640 g) at 65° to -70° C. Then a solution of 4 g sodium carbonate in water (306 g) was added, the apparent pH being 7, and then 5.5 at the end of the treatment period of 31 days.

All samples were stored at 40° C. At various time intervals samples containing equivalent to 0.125% w/w polymer were submitted for biocidal testing. Results are shown in Table 6:

Table 6

Time (days) at 40°C	Time for complete kill (minutes) <10 cfu/mL *(Pseudomonas aeruginosa)*		
	Solution A	Solution B	Solution C
0	30	30	30
7	30	60	-
14	-	-	10
31	60	60	10

Example 5

1 g of poly(2-propenal, 2-propenoic acid) was heated in either a dry or a humid, enclosed chamber, both at 60° C, for 3 days. Solutions of the dry polymer and the humidified polymer, respectively were prepared at 0.125% w/w (with correction for moisture content) and submitted for evaluation by the Biocidal Test:

Table 7

	Cfu/mL * *(Pseudomonas aeruginosa)*					
	0 min	5 min	10 min	15 min	30 min	60 min
Polymer (dry)	$4.9x10^9$	-	$7.6x10^5$	$5.9x10^4$	$1.2x10^2$	<10
Polymer (humidified)	$1.1x10^7$	$6x10^6$	$3.4x10^3$	$3.7x10^3$	<10	-

* Colony forming units/mL

The polymers exhibited carbonyl and/or carboxyl absorption in the I.R. between 1700 - 1730 cm^{-1}, carbonyl groups (e.g. with Schiff's reagent) and have M_w = *ca.* 10000 and M_n = *ca.*5000; titration shows carboxyl groups *ca.* 5 mole %. These parameters are similar (but not the same) as those of poly(2-propenal, 2-propenoic acid).

Example 6

In duplicate experiments, a sample of polymer was prepared and then dissolved in ethane-diol, exactly as described in Example 5 of 11686/95. Half of this material was further heated at 80° C for 24 hours (following which, solubility in aqueous media remained incomplete). The samples were compared for antimicrobial activity, using the standard Biocidal Test. Both of the samples treated by heating, ie. super-activation showed a clear enhancement of antimicrobial activity, as shown in Table 8:

Table 8

	Cfu/mL * *(Pseudomonas aeruginosa)*				
Treatment of solution	Initial Count	5 min	10 min	15 min	30 min
(1) None	$4.6x10^5$	$5.7x10^5$	$2.9x10^2$	<10	<10
(2) None	$4.6x10^5$	$4.2x10^5$	$1.5x10^2$	10	<10
(1) 24 hours 80° C	$4.6x10^5$	$3.7x10^5$	<10	<10	<10
(2) 24 hours 80° C	$4.6x10^5$	$8.0x10^5$	<10	<10	<10

* Colony forming units/mL

Example 7

50 g of poly(2-propenal, 2-propenoic acid) was dissolved in PEG200 (640 g) at between 65 to 70° C. Then, an aqueous solution of sodium carbonate (4 g) in water (306 g) was added. The sample was divided and either stood at room temperature or heated at 80° C for 24 hours. The acrolein content of the solution was determined over time, by reverse phase HPLC and results are shown in Table 9:

Table 9

Days stored at 20° C	Acrolein Content (ppm)	
	Superactivated	Not Superactived
0	274	144
7	-	126
16	34	103
30	13	80

Example 8

Solutions of poly(2-propenal, 2-propenoic acid) were prepared as in Example 7 and treated at temperatures of 40, 60, 80, 100 and 115°C for varying time periods. Samples were subjected to the standard Biocidal Test to confirm the increased kill rate and results are shown in Table 10:

Table 10

Super-activation Temperature(°C)	Optimum Time Range (Hours)	Total Kill Time (minutes)
Room Temperature	>1400	<10
40	1400	<10
60	120-170	<10
80	16-24	<10
100	4-7	<10
115	1-3	<10

The amount of time required for super-activation is seen to be inversely proportional to temperature. All solutions of polymers derived from the superactivation process were completely miscible, in all proportions, with aqueous solvents.

Example 9

(a) 540 g of poly(2-propenal, 2-propenoic acid) was dissolved in 2304 g PEG200 at 65° C, prior to mixing with 43.2 g of sodium carbonate in 712 g of water. Then, the solution was heated to 100° C for 4 hours, and 36g sodium lauryl sulphate, 7 g ECOTERIC T20 (non-ionic detergent) and 2 g lemon fragrance were added. The formulation, pH6, was diluted 1:30 with hard water and challenged against *Staphylococcus aureus* (a Gram-positive bacterium, of particular significance regarding infections in hospitals) and *Salmonella choleraesuis* (a Gram-negative bacterium, of particular significance regarding infections in food preparation areas), respectively using the Association of Agricultural Chemists Official Methods of Analysis (1995) 991.47, 991.48, (Hard Surface Carrier Test Method). Results are shown in Table 11:

Table 11

Micro-organism	Positive Tubes	Result
S aureus	2/60	Pass
S. choleraesuis	1/60	Pass

Adjustment of this formulation to higher pHs, increases the antimicrobial activity, as monitored by the Biocidal Test. Results are shown in Tables 12(a) and 12(b):

Table 12(a)

Activity against *Staphylococcus aureus*

Initial Count, 3×10^6 cfu/mL; polymer 350 ppm.

pH	10 minutes cfu/mL	20 minutes cfu/mL	30 minutes cfu/mL	45 minutes cfu/mL	60 minutes cfu/mL
5.6	2.8×10^5	4.4×10^4	2.3×10^3	20	<10
7.2	2.7×10^3	<10	<10	<10	<10
8.9	3.2×10^3	<10	<10	<10	<10
10.5	1.1×10^2	<10	<10	<10	<10

Table 12(b)

Activity against Pseudomonas aeruginosa

Initial Count, 3.7×10^6 cfu/mL; polymer 350 ppm.

pH	10 minutes cfu/mL	20 minutes cfu/mL	30 minutes cfu/mL	45 minutes cfu/mL	60 minutes cfu/mL
5.6	2.9×10^5	8.6×10^4	6.2×10^2	40	<10
7.2	5.8×10^5	9.1×10^4	4.3×10^3	<10	<10
8.9	9.5×10^5	8.2×10^4	4.6×10^2	<10	<10
10.5	1.1×10^2	3.0×10^3	<10	<10	<10

(b) 1200 g of poly(2-propenal, 2-propenoic acid) was dissolved in 7680 g of PEG200 at 60^0 C and then 96g Na_2CO_3 in 3024 g water was added. The solution was heated at 100°C for 6 hours.

The formulation was added to the basin of an induced draft cooling tower, to a concentration of 300ppm (30ppm polymer) 3 times/week. Dosing was carried out at evening to allow contact times of 8-12 hours before operation recommenced; residual concentration was expected to be halved every 3-6 hours of operation. Recirculation water had on average, temperature 27° C, pH 8.5, conductivity 3000 μS. Microbial counts were determined and compared to an adjacent, identical, tower which was dosed with a biodispersant, daily. Results are shown in Table 13:

Table 13

	Cfu/mL*	
Treatment Time (days)	Treated Tower	Control Tower
1	2.4×10^3	1.1×10^7
2	2.0×10^3	1×10^6
3	3.3×10^3	-
4	2.5×10^3	-
14	6.1×10^4	2.6×10^6
15	5.1×10^4	1.1×10^6
16	5.1×10^4	4.9×10^5

* Colony forming units/mL

The data indicate the treatment programme maintained the microbial counts within the guidelines of AS/NZ Standard 3666.3 (Int):1998 and below that in the adjacent tower, containing biodispersant (which was found to be unusually inadequate during the demanding conditions of the very hot, summer period of the test).

Example 10

10(a) Comparative Example

This example demonstrates a method of preparing an acrolein polymer in which the method of the invention is not used.

1.0 0.8% w/w Sodium Hydroxide

Place 9.90 kg of deionised water in a 10L stainless steel vat and add 0.08 kg sodium hydroxide to the water and stir until dissolved.

2.0 Polymerisation

Place 100.1 kg of deionised water in a 200L stainless steel vat and add 4.99 kg of the 0.8% w/w sodium hydroxide solution to the 200L vat. Equilibrate the solution to 15 – 20° C. Simultaneously add 20 kg acrolein monomer and the remaining 0.8% w/w sodium hydroxide solution to the 200L vat at a rate over 1 hour such that the pH remains at 10.5 –11.0, and the temperature does not rise above 30°C. Continue the polymerisation for a further 90 minutes.

3.0 Washing

Filter/centrifuge the polymerisation mixture and wash the polymer with deionised water until the pH of the wash water is less than 7.0. The approximate yield is 8 kg.

4.0 Drying

Dry the polymer in air, then heat in an oven using the following program.

Step	Time	Temperature
1	2 hrs	25° C
2	1 hr	40° C
3	1 hr	70° C
4	1 hr	75° C
5	2 hrs	85° C

5.0 Dissolution

Place 400L of water in a 500L vat and add 4 kg of sodium carbonate and stir until dissolved. Slowly add 8 kg of dry, heated polymer and stir for thirty minutes.

The resulting polymer was found to have an approximate solubility of 90 – 95% w/w in 1% w/w sodium carbonate.

Example 10(b)

This example describes a method of preparing an acrolein polymer in which the polymer of comparative example is super activated by the method of the invention.

1.0 Base Manufacture

Dissolve 0.4 kg sodium carbonate in 30.6 kg water in a suitable container and place 64 kg polyethylene glycol 200 into the mixing vessel. Commence stirring with the mechanical stirrer and heat the PEG200 to 65 ±3° C. Add 5 kg of the dry acrolein polymer from Example 10a to the PEG200 and stir until a uniform mixture is obtained.

NOTE: The solid may not completely dissolve at this stage.

Slowly add the sodium carbonate solution to the glycol mixture at a rate that ensures the pH of the solution remains in the range 3.5 – 9.0.

Stir the solution for 45 minutes at 65 ±3° C.

NOTE: pH should be within the range 7-9. Temperature should be within the range 65 ±3° C.

2.0 Superactivation

Cover the mixing vessel and heat to 100° C for four (4) hours. The resulting polymer was found to have an approximate solubility 99.5 – 100% w/w in water.

Example 11

This example examines the antimicrobial activity of the dry, normally activated poly(2-propenal, 2-propenoic acid) polymer of Example 10a and the antimicrobial activity of the superactivated acetal derivative described in Example 10b.

Chickens treated each of the antimicrobials were compared with a control group according to the following procedure:

In each trial 20 Cob chickens (Line 53), day old were purchased from a commercial hatchery. They were weighed, sexed and randomly assigned into adjacent pens in a room of an isolated animal house. There was an even distribution of male and female chickens. Water and feed were available ad libitum. The diet was a commercial crumble (Chick Starter, Milne Feeds: 18% crude protein) with a coccidiostat present (125 ppm Dinitolmide).

Ten chickens were administered the formulation of 0.1% w/w of normally activated antimicrobial from Example 10a in the water for 14 days through static drinkers; dose rate of 30 mg/kg/day. The other ten chickens were the Control Group.

Both groups of chickens were weighed on days 0, 4, 7, 11 and 14. At the completion of the trial all chickens were euthanised, and the treated chickens were autopsied post mortem. A thorough gross examination of the thoracic and abdominal cavities was performed.

RESULTS:

Table 14a

Weight gained during trial with normally activated antimicrobial of Example 10a

Day	Control Group (Average Weight in g)	Treatment Group (Average Weight in g)	Differences between groups (%)
0	42.5	42.5	0
4	65.0	72.5	11.5
7	98.5	98.0	-0.5
11	145.5	148.5	2.4
14	185.5	200.5	8.1

The treatment group had measurably greater weight gains at the end of the 14-day period in comparison to the control group.

At the completion of the trial, at post-mortem, no clinical or pathological signs of toxicity were evident at this gross examination in the treated group of chickens.

Table 14b

Weight gains during trial with superactivated acetal antimicrobial of Example 10b

Day	Control Group (Average Weight in g)	Treatment Group (Average Weight in g)	Differences between groups (%)
0	42.5	42.5	0
4	62.5	67.5	8
7	97.5	103	6
11	130	145	11.5
14	178	219	23

At post mortem at the completion of the trial, on the remaining chickens, no clinical or pathological signs were evident at gross examination in both groups.

CONCLUSION:

There was a significant difference in weight gains in the treatment group in comparison to the control group (χ^2; $P<0.015$). The treatment group was 23% heavier than the control group at the completion of the trial.

The significant improvement in weight gain of the superactivated acetal derivative relative to the control, and in the next example, when compared with the normally activated poly(2-propenal, 2-propenoic acid) demonstrates the significant improvement in enteric antimicrobial activity of the acetal derivative.

Example 12

This example evaluates the polymeric antimicrobial of Example 10b under field conditions for the control of porcine post weaning colibacillosis (PWC).

METHOD:

146 Young pigs [weaners], either receiving various formulations of superactivated antimicrobial in their feed or water, APRALAN® (Elanco) orally, autogenous vaccination, or neither, were challenged with the stresses

associated with weaning on a large commercial piggery that had a long history of problems with PWC.

Their responses in the development of diarrhoea, weight gains, and mortality were assessed and are recorded in Table 15.

Table 15

	Superactivated polymeric antimicrobial group number [1]	Mortality Rates [% died][2]	Diarrhoea days [Days loose: score 1-2][3]	Mean Faecal Score[4]
Superactivated polymeric antimicrobial	1	3.33	1.79 [F;P<0.0001]	0.14 [F;P<0.0001]
	2	0	1.17 [F;P<0.0001]	0.11 [F;P<0.0001]
Untreated control group	3	16.67 [F;P<0.05]	3.77 [F;P<0.0001]	0.41 [F;P<0.0001]
Apralan® group	4	13.33 [F;P<0.05]	3.89 [F;P<0.0001]	0.38 [F;P<0.0001]
Vaccinated Group	5	6.67	3.10 [F;P<0.0001]	0.30 [F;P<0.0001]

Notes:

1. Coding of treatments:
   i. Group 1 = 0.1% w/w superactivated polymeric antimicrobial in feed
   ii. Group 2 = 0.02% w/v superactivated polymeric antimicrobial in water
2. Percentage of group that died from PWC during the trial
3. The mean number of days for each pig in the group when a faecal score of 1 or 2 was recorded; faecal score is a measure of the intensity of diarrhoea.
4. The sum of the faecal scores divided by the number of observed samples per pig.
5. F = Fisher's Exact Test.

CONCLUSION:

This field trial highlighted the following points that positively reflected on the efficacy of the acetal derivative of poly(2-propenal, 2-propenoic acid) (superactivated antimicrobial) for use in piglets in commercial piggery, when challenged with β-haemolytic *Escherichia coli* after weaning:

1. Mortality:
   Lower mortality rates for either groups treated with superactivated polymeric antimicrobial than either the untreated, vaccinated or Apralan® groups.

2. Diarrhoea days:
   Significantly lower diarrhoea days in either of the superactivated antimicrobial treated groups [F; P<0.0001] than in either of the untreated, vaccinated or Apralan® groups.

3. Faecal scores:
   Significantly lower mean faecal scores in either of the superactivated antimicrobial treated groups [F; P<0.0001] than in either of the untreated, vaccinated or Apralan® groups.

Example 13

This example examines the effect of using certain additives with the antimicrobial of the invention.

METHOD:

Overnight broths of challenge cultures were prepared and the Total Viable Count (TVC) of the overnight cultures was estimated.

The samples were serially diluted 1 to 1 using sterile normal saline (5mL). Sterile Hard Water (SHW) was used as diluent when testing samples containing EDTA.

1 part of each overnight culture was diluted in 9 parts diluent. The resulting diluted suspensions were used as the inocula.

Inoculate each sample dilution with 100 µL of the diluted overnight culture. (One culture per tube). Mix well.

The tubes were incubated for ≤ 24 hours at 37° C (*A. niger* was incubated at 28°C for ≤ 24 hours).

From each test tube, 1 mL was subcultured into 9 mL of recovery broth and mixed well (Recovery Broths: Nutrient Broth + 3% Tween 80 (NBT) for polymeric antimicrobial and EDTA; Nutrient Broth + 3% Tween 80 + 0.1% ammonia (NBTA) for glutaraldehyde and, Letheen Broth (LB) for methyl parabens).

The samples incubated at 37° C for a further ≤ 48 hours. (28°C for ≤ 5 days for *A niger).*

Each tube was examined for growth. All tubes were then subcultured onto selective agar and incubated for ≤ 24 hours at 37° C. (28° C for ≤ 5 days for *A. niger*). Growth on selective agar was taken to confirm growth of the test organism.

Positive controls were performed using 5mL of diluent inoculated with culture and subject to incubation, recovery and confirmation.

Negative controls were performed using un-inoculated 5 mL of diluent and subject to incubation, recovery and confirmation.

RESULTS: Results are tabulated below

Table 16

MKC of Selected Preservatives and Synergy Index

Note: all ratio values are expressed as ratio of polymeric antimicrobial: preservative

Culture	Inoculum (cfu/mL)	1	2	3	4	5	6	7	8	9	10	11
A. niger	3.3×10^7		2000	>250		1000:125						
	3.0×10^7						2000	>1000	1000:500			
	2.2×10^7									2000	10000	500:2500
C .albicans	3.0×10^6		1000	125		250:31						
	3.2×10^6						1000	1000	250:125			
	1.1×10^7									500	1250	125:62.5
E.coli	1.6×10^9	62.5		3.5	3.5							
	1.0×10^9						125	>1000	62.5:31			
	9.6×10^8									62.5	2500	62.5:310
P.aeruginosa	1.9×10^9	250		31	31							
	9.4×10^8						250	1000	125:62.5			
	1.8×10^9									250	1250	62.5:310
S.aureus	1.5×10^9	31		7	3.5							
	1.3×10^9						31	>1000	31:15			
	1.3×10^9									31	2500	31:150

Legend:

1) Polymeric antimicrobial 0.025% w/w
2) Polymeric antimicrobial 0.2% w/w
3) Glutaraldehyde 0.025% w/w
4) Polymeric antimicrobial 0.025% w/w + Glutaraldehyde 0.025% w/w
5) Polymeric antimicrobial 0.2% w/w + Glutaraldehyde 0.025% w/w
6) Polymeric antimicrobial 0.2% w/w
7) EDTA 0.1% w/w
8) Polymeric antimicrobial 0.2% w/w + EDTA 0.1% w/w
9) Polymeric antimicrobial 0.2% w/w (in 80% w/w glycerol)
10) Methyl Paraben 1% w/w (in 80% w/w glycerol)
11) Polymeric antimicrobial 0.2% w/w (in 80% w/w glycerol) + Methyl Paraben 1% w/w (in 80% w/w glycerol).

Table 17

Synergy Index with polymeric antimicrobial

Culture	Glutaraldehyde	EDTA	Methyl paraben
A.niger	<0.6	<0.5	0.2
C.albicans	0.3	0.1	0.5
E.coli	0.5	<0.3	0.3
P.aeruginosa	0.6	0.4	0.2
S.aureus	0.3	<0.7	0.2

Note: Synergy <1.0, Additive = 1.0, Antagonistic >1.0

Whereby SI=CD/A + CE/B

A= MKC polymeric antimicrobial

B= MKC Preservative

C= MKC Mix

D= Ratio of A to B

E= Ratio of B to A

CONCLUSION:

It was shown that the acetal derivative of poly(2-propenal, 2-propenoic acid) was synergistic with Glutaraldehyde, EDTA, and Methyl Paraben, respectively versus A. niger, C. albicans, E. coli, P. aeruginosa, S. aureus.

Example 14

This example demonstrates the activity of the antimicrobial of the invention in combination with "Dettol" brand antimicrobial.

The sample was serially diluted 1 to 1 using sterile normal saline (5mL).

Each sample dilution was inoculated with 100µL of the diluted overnight culture. (One culture per tube). The samples were mixed well.

They were incubated at 37 ± 2oC for ≤ 24 hours. (*Aspergillus niger* inoculated tubes were incubated at 28 ± 2°C for ≤ 24 hours).

From each test tube 1mL was subcultured into 9mL recovery broth and vortexed well (NBT, or Sabouraud + 3% Tween 80 (SABT) for *A. niger*).

They were incubated at 37 ± 2oC for ≤ 48 hours. (*A. niger*, was incubated at 28 ± 2° C for a further 5 days).

The recovery broths were examined for turbidity (Growth) and streaked onto selective agars to confirm growth.

Table 18

MKC Results in ppm of superactivated polymeric antimicrobial and/or "Dettol"

Culture	Inoculum Approx (cfu/mL)	Polymeric Antimicrobial MKC range (ppm)	1	2	3	4	5
A. niger	$4.1x10^7$	2000 – 500	N/A	1000	300	N/A	125:150
C. albicans	$5.2x10^6$	100 – 250	N/A	500	75	N/A	62.5:75
E. coli	$7.6x10^8$	125 – 31	125	N/A	75	31:75	N/A
P.aeruginosa	$1.8x10^9$	250 - 62.5	N/A	250	600	N/A	125:150
S. aureus	$2.0x10^9$	31 – 7	N/A	15	75	N/A	15:19

Legend

1) 0.1% w/w polymeric antimicrobial
2) 0.2% w/w polymeric antimicrobial
3) "Dettol" 4.8% w/v (Diluted 1:20)
4) 0.1% w/w polymeric antimicrobial + "Dettol" (Diluted 1:20)
5) 0.2% w/w polymeric antimicrobial + "Dettol" (Diluted 1:20)

Table 19

Synergy Index of polymeric antimicrobial and Dettol

Culture	Synergy Index (SI)
A. niger	0.3
C. albicans	0.6
E. coli	0.6
P. aeruginosa	0.4
S. aureus	0.6

Note: Antagonistic if SI >1, Additive if SI=1, Synergistic if SI<1

SI= CD/A + CE/B

A= MKC polymeric antimicrobial (ppm)

B= MKC Dettol (ppm)

C= MKC of polymeric antimicrobial / "Dettol" Mix (ppm)

D= Ratio of polymeric antimicrobial in relation to Dettol

E= Ratio of "Dettol" in relation to polymeric antimicrobial

Note: The active antimicrobial in "Dettol" is chloroxylenol

CONCLUSION:

It was shown that the polymeric antimicrobial of the invention was synergistic with "Dettol" against E. coli, S. aureus, P. aeruginosa, C. albicans and A. niger. This shows that "Dettol" and the polymeric antimicrobial when used together as a mixed solution, will work substantially better than when used on their own.

Example 15

Antiseptic Qualities of poly(2-propenal, 2-propenoic acid) [superactivated]

The antimicrobial, poly(2-propenal, 2-propenoic acid) [superactivated], was trailed for antiseptic qualities.

The number of bacteria present on the hands of subjects was determined before and after application of antimicrobial followed by donning of surgical gloves. The antiseptic effect of poly(2-propenal, 2-propenoic acid) [superactivated] was compared to the commonly used surgical antiseptic, 4% Chlorhexidine Surgical Scrub (manufactured by Orion Laboratories, in Perth, Western Australia).

- 3% w/w aqueous solutions of poly(2-propenal, 2-propenoic acid) [superactivated] reduced baseline counts of bacterial populations on gloved hands after 3 hours.

- 2% w/w aqueous solution of poly(2-propenal, 2-propenoic acid) [superactivated] with 70% ethanol showed a sustained reduction in baseline bacterial counts after 3 hours on gloved hands, as did 4% chlorhexidine.

- 3.2% w/w aqueous solution of poly(2-propenal, 2-propenoic acid) [superactivated] with 3.1% sodium lauryl sulphate, followed by 4% w/w aqueous solution of poly(2-propenal, 2-propenoic acid) [superactivated] in 70% ethanol, applied to hands prior to donning surgical gloves, gave a significant reduction in the baseline bacterial counts after 3 hours.

The results indicate that poly(2-propenal, 2-propenoic acid) [superactivated] possesses good residual antimicrobial activity that is required for sustained control of bacterial numbers in surgical asepsis. The inclusion of 70% ethanol to the formulation aids the initial rapid decrease in bacterial numbers.

Example 16

The Biocidal Activity of 0.125% w/w and 0.05% w/w Superactivated Polymer against the reference strain *H. pylori* NCTC 11637 at pH 7 and pH 4.

The efficacy *in vitro* of the Superactivated Polymer was first established against the *H. pylori* reference strain, *H. pylori* NCTC11637. As variables, two concentrations were chosen; one was a 40-fold dilution of the 5% solution of the Superactivated Polymer prepared in Example 2 giving a 0.125% w/w concentration of the Superactivated Polymer, mimicking the dilution in the stomach; the other was a 100-fold dilution giving 0.05% w/w concentration of Superactivated Polymer. Two pHs were chosen, pH 7 as the baseline and pH 4 to mimic conditions in the stomach.

Cultures *H. pylori* NCTC11637 were grown microaerophilically on selective agar plates at 37 ± 2°C until sufficient growth was observed. Growth was aseptically removed from the plates and prepared as a standardized turbid suspension of 10% T, as displayed on a Vitek colorimeter, diluting with sterile normal saline. 19.9g of sample was weighed out and inoculated with 100µL of culture suspension. 1mL of sample was immediately transferred into the Deactivation/Recovery broth (Nutrient Broth plus 3% Tween 80) and then serially diluted. 100µL aliquots were placed onto selective agar plates and spread using a sterile disposable spreader. The transfer steps were repeated at time intervals of 5, 10, 15 and 20 minutes. All plates were incubated microaerophilically at 37 ± 2°C until sufficient growth was achieved (approximately 5 to 7 days). All colonies were counted and the population decline over time was determined. The test was repeated using sterile normal saline as the sample to determine the natural die-off rate at atmospheric conditions.

Legend:

Culture 1. Superactivated Polymer, pH 7, 0.125% w/w

Culture 2. Superactivated Polymer, pH 7, 0.05% w/w.

Culture 3. Superactivated Polymer, pH 4, 0.125% w/w.

Culture 4. Superactivated Polymer, pH 4, 0.05% w/w.

Culture 5. Sterile Normal Saline, pH 7.

Culture 6. Sterile Normal Saline, pH 4.

Table 20

Biocidal Activity of CHEMEQ^{RTM} polymeric antimicrobial on *H. pylori* (NCTC 11637)

Culture	T = 0 min	T = 5 min	T = 10 min	T = 15 min	T = 20 min
1	1.0×10^5	8.6×10^3	1.0×10^2	$<1.0 \times 10^2$	$<1.0 \times 10^2$
2	1.6×10^5	2.0×10^3	4.0×10^2	1.0×10^2	$<1.0 \times 10^2$
3	1.0×10^5	3.2×10^4	1.1×10^4	4.0×10^2	$<1.0 \times 10^2$
4	3.0×10^4	1.7×10^4	1.1×10^4	8.2×10^3	3.6×10^3
5	2.0×10^5	1.0×10^5	9.2×10^5	8.0×10^4	7.9×10^4
6	2.0×10^4	1.4×10^4	8.0×10^3	6.0×10^3	6.0×10^3

NOTE: Counts in Colony Forming Units (cfu) per mL of Deactivation Broth

The results against the reference strain (Table 20) show that the Superactivated Polymer was effective at pH 7 at both 0.125% w/w and 0.05% w/w, and was also effective at pH 4 and 0.125% w/w.

Example 17

Further to the analysis of Example 16, three additional strains of *H. pylori* at pH 7 and pH 4 were examined: *H. pylori* 01/303, which is resistant to clarithromycin and metronidazole; *H. pylori* SS1, a clinical strain isolated in Sydney with a high colonising ability of interest for possible animal models and *H. pylori* ATCC 700392, a strain whose genome has been sequenced and comes from the UK.

Table 21

Biocidal Activity of Superactivated Polymer at 0.125% pH 7

Culture	T = 0 min	T = 5 min	T = 10 min	T = 15 min	T = 20 min
Control H. pylori 11637	2.0 × 105	1.0 × 105	9.2 × 105	8.0 × 104	7.9 × 104
H. pylori 11637	1.0 × 105	8.6 × 103	1.0 × 102	<1.0 × 102	<1.0 × 102
H. pylori 01/303	3.6 × 105	2.8 × 103	<1.0 × 102	<1.0 × 102	<1.0 × 102
H. pylori SS1	2.8 × 105	1.4 × 103	2.0 × 102	<1.0 × 102	<1.0 × 102
H. pylori 700392	2.0 × 106	1.3 × 106	1.8 × 105	8.8 × 103	<1.0 × 102

When treated with the Superactivated Polymer at 0.125% w/w at pH 7, all strains were rapidly killed, with the antibiotic resistant strain being particularly vulnerable with death coming at less than 10 minutes (Table 21). The control strain was untreated.

Example 18

The method of Example 3 was repeated in testing the Biocidal Activity of 0.125% w/w Superactivated polymer (pH 4) against all strains of *H. pylori*.

Table 22

Biocidal Activity of CHEMEQ^{RTM} polymeric antimicrobial at 0.125% and pH 4.

Culture	T = 0 min	T = 5 min	T = 10 min	T = 15 min	T = 20 min
Control *H. pylori* 11637	2.0×10^4	1.4×10^4	8.0×10^3	6.0×10^3	6.0×10^3
H. pylori 11637	1.0×10^6	3.2×10^4	1.1×10^4	4.0×10^2	$<1.0 \times 10^2$
H. pylori 01/303	4.2×10^6	1.1×10^6	5.0×10^4	2.0×10^4	2.0×10^2
H. pylori SS1	4.9×10^6	1.0×10^6	1.0×10^5	2.8×10^4	2.0×10^2
H. pylori 700392	5.5×10^6	1.9×10^6	1.2×10^6	3.6×10^4	$<1.0 \times 10^2$

Testing the Superactivated Polymer at the stomach mimicking pH of 4 and at 0.125% w/w resulted in all strains being killed within 20 minutes (Table 3). This result is significant because this time frame for killing H. pylori is less than the time for passage through the stomach (40 min –1 hour). This demonstrates the effectiveness of the Superactivated Polymer at a pH, a concentration, and in a time frame consistent with treating an *H. pylori* infection in the stomach. The control strain was untreated.

Example 19

This example demonstrates the enteric antimicrobial activity of the acetal derivative of poly(2-propenal, 2-propenoic acid) prepared according to the procedure of Example 10b.

MATERIAL AND METHODS:

Sixteen weaner pigs (age: 18 days ±2 days and weight: 5.5 kg ±1.0 kg) were purchased from a commercial piggery. They were randomly assigned into 2 groups of 8 pigs (equal distribution of sexes) and housed in an environmentally controlled isolation animal house.

Water and feed were available ad libitum upon entry to the animal house, and the diet was a commercial antimicrobial-free weaner pellet [19% crude protein].

All the weaner pigs were euthanised with an intravenous injection of sodium barbiturate, and then necropsied. DNA from Gastric and Oesophageal regions of the stomach of twenty-four weaner pigs were extracted at necropsy using a Qiagen Dneasy tissue kit according to provided instructions. 3µL of the extracted DNA was used to test for the presence of *Helicobacter spp.* in the biopsy tissue samples. Polymerase Chain Reaction (PCR) was conducted twice on each sample with seven control DNA samples being included in each PCR run. No discrepancies were found between the PCRs conducted.

RESULTS:

Coding of treatments:

Group 1: No treatment (negative control)

Group 2: 0.1% w/v of superactivated polymeric antimicrobial according to Example 10b; 30 mg/kg/day.

Table 23

PCR results of *Helicobacter spp.* using previously optimised genus specific primers where + represents a positive detection of *Helicobacter spp.* and – represents no detection.

Group Number	Gastric Region	Oesophageal Region
1	-	-
1	-	+
1	-	+
1	+	-
1	-	-
1	-	+
1	-	-
1	-	+
2	-	-
2	-	-
2	-	-
2	-	-
2	-	-
2	-	-
2	-	-
2	-	-

In group 1 (no treatment) there were five positive PCR results to *Helicobacter spp* (1 – Gastric, 4 – Oesophageal), while in group 2 (0.1% w/v of polymeric antimicrobial) there were no positive PCR results.

CONCLUSION:

The acetal derivative of poly(2-propenal, 2-propenoic acid) at 0.1% w/v significantly (χ^2: $P<0.025$) reduces the incidence of porcine *Helicobacter spp* in the gastric and oesophageal mucosa in weaner pigs.

Example 20

Comparative Example 20(a)

This example demonstrates a method of preparing non-superactivated poly(2-propenal, 2-propenoic acid).

0.8% w/w Sodium Hydroxide

Place 9.90 kg of deionised water in a 10L stainless steel vat and add 0.08 kg sodium hydroxide to the water and stir until dissolved.

Polymerisation

Place 100.1 kg of deionised water in a 200L stainless steel vat and add 4.99 kg of the 0.8% w/w sodium hydroxide solution to the 200L vat. Equilibrate the solution to 15 – 20° C. Simultaneously add 20 kg acrolein monomer and the remaining 0.8% w/w sodium hydroxide solution to the 200L vat at a rate over 1 hour such that the pH remains at 10.5 –11.0, and the temperature does not rise above 30° C. Continue the polymerisation for a further 90 minutes.

Washing

Filter/centrifuge the polymerisation mixture and wash the polymer with deionised water until the pH of the wash water is less than 7.0. The approximate yield is 8 kg.

Drying

Dry the polymer in air, then heat in an oven using the following program.

Step	Time	Temperature
1	2 hrs	25° C
2	1 hr	40° C
3	1 hr	70° C
4	1 hr	75° C
5	2 hrs	85° C

Dissolution

Place 400L of water in a 500L vat and add 4 kg of sodium carbonate and stir until dissolved. Slowly add 8 kg of dry, heated polymer and stir for thirty minutes.

The resulting polymer was found to have an approximate solubility of 90 – 95% w/w in 1% w/w sodium carbonate.

Example 20(b)

This example describes a method of preparing an acrolein polymer in which the polymer of comparative example 20(a) is super activated

Base Manufacture

Dissolve 0.4 kg sodium carbonate in 30.6 kg water in a suitable container and place 64 kg polyethylene glycol 200 into the mixing vessel. Commence stirring with the mechanical stirrer and heat the PEG200 to 65 ±3° C. Add 5 kg of the dry acrolein polymer from Example 20(a) to the PEG-200 and stir until a uniform mixture is obtained.

NOTE: The solid may not completely dissolve at this stage.
Slowly add the sodium carbonate solution to the glycol mixture at a rate that ensures the pH of the solution remains in the range 3.5 – 9.0.

Stir the solution for 45 minutes at 65 ±3° C.

NOTE: pH should be within the range 7-9. Temperature should be within the range 65 ±3° C.

Superactivation

Cover the mixing vessel and heat to 100° C for four (4) hours. The resulting Superactivated Polymer was found to be miscible with water in all proportions.

Example 21

In Example 14 of PCT/AU9600328, it was demonstrated that poly(2-propenal,2-propenoic acid) polymer in 0.5% w/w sodium carbonate solution possessed anticancer activity against the Ehrlich ascites cell line in a mouse model.

The anticancer activity of poly(2-propenal,2-propenoic acid) polymer [Example 20(a)] against that of the Superactivated Polymer [Example 20(b)]. An *in vitro* model of a gastrointestinal cancer was carried out on the human colon cancer cell line, HT-29. Poly(2-propenal, 2-propenoic acid) was used in a 5% w/w concentrate. The test used incubates the cancer cells with varying concentrations of polymer to give a plot from which an IC_{50} can be established.

Methodology

HT-29 cells (human colon cancer cells) were seeded (in 100 µl) into the wells of 96-well culture plates and incubated overnight at 37°C in a humidified 5% CO_2, 95% air atmosphere. The polymer [poly(2-propenal,2-propenoic acid) polymer in Comparative Example 20(a) and the Superactivated Polymer in Example 20(b)] was dissolved in water and then diluted in medium to 10 concentrations spanning a 4-log range. 100 µl of each solution was then added to each of 5 wells. The plates were incubated for a further 72 hr after which viable cells were measured using the sulforhodamine B assay (Skehan *et al.*, (1990) J. Nat. Cancer Inst. *82*: 1107-1112.; Monks *et al.*, (1991) J. Nat. Cancer Inst. *83*: 757-766.). The cells were then fixed with 10% cold trichloroacetic acid for 1 hr at 4°C and the plates rinsed with distilled water, left to air dry and then stained with 0.4% sulforhodamine B (Aldrich) in 1% acetic acid (v/v) for 30 min. Unbound dye is then removed by washing twice with distilled water and finally with 1% acetic acid. Protein-bound dye is then solubilized in 10 mM unbuffered Tris base and the absorbance read at 550 nm using an automatic plate reader. The mean absorbance for each drug dose is expressed as a percentage of the control untreated well absorbance.

The results of the test are shown in Table 24.

The poly(2-propenal,2-propenoic acid) polymer of Comparative Example 20(a) gave an average IC_{50} over two tests of 0.030%; the poly(2-propenal,2-propenoic acid) polymer being assigned the value of 100%. This translates to 0.0015% w/w of active polymer.

The Superactivated Polymer in Example 20(b) gave an average IC_{50} over four tests of 0.025%. This translates to 0.00125% w/w of the Superactivated Polymer and indicates that the Superactivated Polymer has potent anticancer activity.

Table 24

IC_{50} of CHEMEQ^{RTM} polymeric antimicrobial against HT-29 human colon cancer cells.

Compound tested	Drug exposure (hrs)	IC_{50} (%)	Average IC_{50} (% w/w)	Average IC_{50} (as % w/w of active polymer)
Comparative Example 20(a)	72	0.037, 0.023	0.030	0.0015
Example 20(b)	72	0.017, 0.034, 0.025, 0.023	0.025	0.00125

IC_{50} is the concentration required to inhibit cell growth by 50%.

Finally, it is understood that various other modifications and/or alterations may be made without departing from the spirit of the present invention as outlined herein.

CLAIMS

1. A method for treatment or prophylaxis of gastrointestinal disease in an animal (including human) comprising gastrointestinal administration to the animal of an effective amount of a polymer comprising a derivative of poly(2-propenal, 2-propenoic acid) formed by reaction between a poly(2-propenal, 2-propenoic acid) and an organic compound containing one or more hydroxyl groups to form protected carbonyl groups.

2. A method according to claim 1 wherein the polymer is orally administered.

3. A method according to claim 1 wherein the animal is suffering from at least one gastrointestinal disease selected from the group consisting of gastroenteritis, ulcer, diarrhoea and gastrointestinal cancer and dysentery-promoted insufficient weight gain.

4. A method according to claim 1 wherein the animal is suffering from at least one of diarrhoea, gastroenteritis and dysentery.

5. A method according to claim 1 wherein the animal is selected from the group consisting of dogs, pigs, sheep, horses, cattle, cats, poultry, ducks, turkeys and quail.

6. A method according to claim 1 wherein the animal is selected from ruminant animals and the polymer is rectally administered.

7. A method according to claim 1 wherein the animal is selected from poultry and pigs.

8. A method according to claim 1 wherein the animal is a partially grown pig.

9. A method for treatment or prophylaxis of porcine post weaning colibacillosis comprising orally administering to young pigs after weaning, an antimicrobially effective amount of the polymer of claim 1.

10. A method according to claim 1 wherein the antimicrobial of claim 1 is administered at a dose of from 0.05 to 5000 mg/kg/day.

11. A method according to claim 1 wherein the antimicrobial of claim 1 is administrated at a dose in the range of from 0.5 to 500 mg/kg/day.

12. A method according to claim 1 wherein the young pigs are administered a dose of the antimicrobial in the range of from 0.05 to 50 mg/kg/day.

13. A method according to claim 1 wherein the gastrointestinal disease results from one or more microbes selected from the group consisting of Coliforms, *Salmonella, P.aeruginosa, Helicobacter, Proteus, Enterobacteria, Yeasts, Protozoa, Clostridia, Shigella* and *Coccidia*

14. A method for the treatment or prophylaxis of diseases of the gastrointestinal tract caused by *Helicobacter* infection comprising the gastrointestinal administration of a therapeutic amount of a polymer comprising a derivative of poly(2-propenal, 2-propenoic acid) formed by the reaction between a poly(2-propenal, 2-propenoic acid) and an organic compound containing hydroxyl groups selected from alkanols, phenols, polyols and mixtures thereof, to form protected carbonyl groups.

15. A method according to claim 1 wherein the gastrointestinal disease results from at least one of enterotoxigenic *E. coli* and β-haemolytic *E. coli.*

16. A method of treatment or prevention of necrotic enteritis in poultry comprising administering to poultry an effective amount of the antimicrobial of claim 1.

17. A method according to claim 1 wherein the antimicrobial is administered in combination with a further chemotherapeutic adsorbed thereon to thereby reduce membrane penetration of the further chemotherapeutic.

18. A method of treatment or prevention of coccidiosis in poultry comprising administering to poultry an antimicrobially effective amount of antimicrobial of claim 1.

19. A method according to claim 1 wherein the derivative comprises a multiplicity of protected carbonyl groups selected from at least one of hemiacetal groups and acetal groups.

20. A method according to claim 20 wherein the protected carbonyl groups include acetal groups.

21. A method according to claim 1 wherein the alcohol is selected from alkanols, phenols, polyols and mixtures thereof.

22. A method according to claim 22 wherein the alcohol is selected from at least one polyol.

23. A method composition according to claim 23 wherein the polyol comprises a polyalkylene glycol.

24. A method according to claim 23 wherein the polyol comprises a polyethylene glycol.

25. A method according to claim 23 wherein the polyol is a polyethylene glycol of molecular weight of from 200 to 2000.

26. An antimicrobial for treating or preventing gastrointestinal disease in animals by gastrointestinal administration said antimicrobial composition comprising a derivative of poly(2-propenal, 2-propenoic acid) formed by reaction between a poly(2-propenal, 2-propenoic acid) and an organic compound

containing one or more hydroxyl groups to form protected carbonyl groups and a pharmaceutically or veterinarially acceptable inert carrier for gastrointestinal administration to animals.

27. An antimicrobial for treating or preventing gastrointestinal disease according to claim 27 wherein the carrier for gastrointestinal administration is selected from the group consisting of water, controlled release polymers, olive oil, peanut oil, sesame oil, sunflower oil, arachis oil, coconut oil, liquid paraffin, ethylene glycol, propylene glycol, polyethylene glycol, ethanol, propanol, isopropanol, glycerol, fatty alcohols, triglycerides, polyvinyl alcohol, partially hydrolysed polyvinylacetate and mixtures thereof.

28. An antimicrobial composition according to claim 27 in the form of a feed additive or drinking water additive comprising from 0.1 to 70% by weight of the antimicrobial according to claim 1.

29. An animal (including humans) feed or drinking water composition comprising a feed material or water and an antimicrobially effective amount of an antimicrobial according to claim 1.

30. An animal feed composition according to claim 30 wherein antimicrobial is present in an amount of from 0.001 to 25% by weight of the total feed or water composition.

31. An antimicrobial composition comprising an antimicrobial according to claim 1 and a further active agent selected from the group consisting of antimicrobials and chemotherapeutic agents.

32. A composition according to claim 31 wherein the further antimicrobial comprising (on a weight basis of the composition) at least one of

(a) a phenol in an amount of 0.1 to 10%;

(b) an isothiazolinone in an amount of 0.001 to 1%;

(c) alkyl parabens in an amount of 0.02 to 2% and

(d) lower alkanol in an amount of from 20 to 99.9%.